A porcine reproductive and respiratory syndrome virus candidate vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting against homologous virus challenge

A porcine reproductive and respiratory syndrome virus candidate vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting against homologous virus challenge

D. Evenson[a], P.F. Gerber[b], C.T. Xiao[a], P.G. Halbur[a], C. Wang[a], D. Tian[c], Y.Y. Ni[c], X.J. Meng[c], and T. Opriessnig[a,b,*]

[a]*Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA*

[b]*The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK*

[c]*Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*

Running title: Efficacy of the SAVE approach in protecting against PRRSV

*Corresponding author. Tel.: +44 (0)131 651 9177. E-mail address: Tanja.Opriessnig@roslin.ed.ac.uk (T. Opriessnig).

Keywords: Porcine reproductive and respiratory syndrome virus (PRRSV); Attenuation; Synthetic attenuated virus engineering (SAVE); Vaccine; Efficacy.

Word count abstract: 296/300

Word count main manuscript text: 2986/3000

Abstract

Current porcine reproductive and respiratory syndrome virus (PRRSV) vaccines sometimes fail to provide adequate immunity to protect pigs from PRRSV-induced disease. This may be due to antigenic differences among PRRSV strains. Rapid production of attenuated farm-specific homologous vaccines is a feasible alternative to commercial vaccines. In this study, attenuation and efficacy of a codon-pair de-optimized candidate vaccine generated by synthetic attenuated virus engineering approach (SAVE5) were tested in a conventional growing pig model. Forty pigs were vaccinated intranasally or intramuscularly with SAVE5 at day 0 (D0). The remaining 28 pigs were sham-vaccinated with saline. At D42, 30 vaccinated and 19 sham-vaccinated pigs were challenged with the homologous PRRSV strain VR2385. The experiment was terminated at D54. The SAVE5 virus was effectively attenuated as evidenced by a low magnitude of SAVE5 viremia for 1-5 consecutive weeks in 35.9% (14/39) of the vaccinated pigs, lack of detectable nasal SAVE5 shedding and failure to transmit the vaccine virus from pig to pig. By D42, all vaccinated pigs with detectable SAVE5 viremia also had detectable anti-PRRSV IgG. Anti-IgG positive vaccinated pigs were protected from subsequent VR2385 challenge as evidenced by lack of VR2385 viremia and nasal shedding, significantly reduced macroscopic and microscopic lung lesions and significantly reduced amount of PRRSV antigen in lungs compared to the non-vaccinated VR2385-challenged positive control pigs. The nasal vaccination route appeared to be more effective in inducing protective immunity in a larger number of pigs compared to the intramuscular route. Vaccinated pigs without detectable SAVE5 viremia did not seroconvert and were fully susceptible to VR2385 challenge. Under the study conditions, the SAVE approach was successful in attenuating PRRSV strain VR2385 and protected against homologous virus

45 challenge. Virus dosage likely needs to be adjusted to induce replication and protection in a
46 higher percentage of vaccinated pigs.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in the global pig population and associated with reproductive failure in adult pigs and respiratory disease in growing pigs [1] resulting in estimated annual losses of $664 million to the U.S. swine industry [2]. PRRSV is an enveloped, positive-sense, single-stranded RNA virus [3;4] that belongs to the family *Arteriviridae* in the order *Nidovirales* [4]. The PRRSV genome contains eight structural protein open reading frames (ORFs) and a few non-structural protein ORFs [5] and isolates can be divided into two main genotypes: type 1 (European type), and type 2 (North American type) [6]. Mutations such as insertions or deletions occur frequently in the PRRSV genome making it one of the most genetically diverse pig viruses [7].

The enormous continual change and diversity of PRRSV strains has resulted in limited efficacy of current commercial vaccines and vaccination strategies [8]. Modified live-attenuated virus vaccines (MLVs) are the most effective option currently available to control clinical signs associated with PRRSV infection; however, while these vaccines in general protect pigs well against homologous challenge they are not always capable of eliciting protective immunity against heterologous field strains [9]. Due to limited vaccine homology with circulating field strains on some farms, pig producers often rely on planned exposure to the pathogenic farm strain [10] which is risky and may not always be economically beneficial or acceptable from an animal welfare point of view. Therefore, rapid attenuation of PRRSV would afford an opportunity to quickly generate farm-specific vaccines.

Recently the synthetic attenuated virus engineering (SAVE) approach was utilized to rapidly attenuate the wild-type PRRSV isolate VR2385 [11]. Specifically, the codon-pairs of the major envelope GP5 gene of PRRSV were deoptimized through a computer algorithm which

resulted in a modified GP5 nucleotide sequence while retaining the original amino acid sequence. The resulting virus was designated SAVE5. When SAVE5 was tested *in vitro* it was genetically stable. Experimental infection of pigs resulted in lower levels of viremia and reduced macroscopic and microscopic lung lesions compared to the wild-type VR2385 virus [11]. The protective efficacy of the SAVE5 was unknown. In the present study, the immunogenicity and protective efficacy of SAVE5 in decreasing clinical signs, lesions and viremia associated with wild-type PRRSV challenge were assessed using a conventional pig model.

2. Methods

2.1. Animals and housing

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (14-D-0008-A). Sixty-eight, 2-week-old, commercial crossbred pigs from a PRRSV-free source herd were transported to the Livestock Infectious Disease Isolation Facility at Iowa State University, Ames, Iowa, USA. The study was done in two replicates 12 months apart. The same source herd was used for both replicates. Upon arrival, the pigs were randomly assigned to one of five groups. Initially and for each replicate, vaccinated pigs were housed in one room and the sham-vaccinated pigs were housed in another room. Prior to virus challenge, the pigs were further separated by treatment status into four (Replicate 1) or three (Replicate 2) rooms with one pen in each room. Pigs were given continuous access to age appropriate feed (Nature's Made, Heartland Co-op, Cambridge, Iowa, USA) and water.

2.2. Experimental design

The experimental groups are outlined in Table 1. At day 0 (D0) when the pigs were 3 weeks old, VAC-IM-CONTROL, VAC-IM-PRRSV and VAC-IN-PRRSV were vaccinated with the SAVE5 candidate vaccine and the NEG-CONTROL and POS-CONTROL groups were sham-vaccinated with saline. The intramuscular (IM) route was used for the VAC-IM-CONTROL, VAC-IM-PRRSV, NEG-CONTROL and the POS-CONTROL groups and the intranasal (IN) route was used for the VAC-IN-PRRSV group. At D42, pigs were challenged with the homologous PRRSV strain VR2385 (VAC-IM-PRRSV, VAC-IN-PRRSV, POS-CONTROL) or were sham-inoculated with saline (VAC-IM-CONTROL, NEG-CONTROL). All pigs were necropsied at D54. Blood samples were collected weekly from D0 through D42 and on D44, D46, D48, D51 and D54. The blood was centrifuged at 3000 g for 10 min and the serum was stored at -80°C until testing. Nasal swabs were taken from each pig on D7, D14, D21, D28, D35, D42, D44, D46, D48, D50 and D53, placed into 1 ml of saline and stored at -80°C until testing. The pigs were weighed on D0, D42 and D54.

2.3. Vaccination

A previously described vaccine candidate, the SAVE5 virus, was utilized [11]. At 3 weeks of age, the VAC-IM-CONTROL and the VAC-IM-PRRSV groups received 3 ml of SAVE5 virus at a dose of $10^{4.5}$ $TCID_{50}$/ml intramuscularly into the right neck. The VAC-IN-PRRSV group received 3 ml of SAVE5 virus at a dose of $10^{4.5}$ $TCID_{50}$/ml intranasally by slowly dripping the inoculum into the nostrils. The NEG-CONTROL and the POS-CONTROL groups received 3 ml of saline intramuscularly into the right neck.

2.4. Challenge

115 At 9 weeks of age (D42 post-vaccination), POS-CONTROL, VAC-IM-PRRSV and
116 VAC-IN-PRRSV groups were challenged intranasally with $10^{6.6}$ $TCID_{50}$ of PRRSV VR2385 by
117 slowly dripping 1 ml of inoculum into each nostril. The NEG-CONTROL and the VAC-IM-
118 CONTROL groups were similarly sham-challenged with 1 ml of saline.
119
120 *2.5. Clinical evaluation*
121 On D42, D44, D46, D48, D50 and D53, rectal temperatures were taken and respiratory
122 scores were evaluated [12]. The respiratory scores included 0=normal, 1=mild
123 dyspnea/tachypnea when stressed, 2= mild dyspnea/tachypnea at rest, 3= moderate
124 dyspnea/tachypnea when stressed, 4= moderate dyspnea/tachypnea at rest, 5= severe
125 dyspnea/tachypnea when stressed and 6= severe dyspnea/tachypnea at rest [12].
126
127 *2.6. Serology*
128 All serum samples were tested for PRRSV specific IgG antibodies using the IDEXX
129 PRRS X3 Ab ELISA (IDEXX Laboratories, Inc.) according to the manufacturer's instructions. A
130 sample-to-positive (S/P) ratio greater than 0.4 was considered positive. A fluorescent focus
131 neutralization (FFN) assay for determination of amount of PRRSV-specific neutralizing
132 antibodies was done on sera collected on D42 according to protocols routinely performed at the
133 Veterinary Diagnostic Laboratory at Iowa State University. The PRRSV strain used was type 2
134 PRRSV strain ISU-P. In addition, FFN on D42 samples were also done at the Animal Disease
135 Research and Diagnostic Laboratory at South Dakota State University using the VR2332 PRRSV
136 type 2 strain. These strains are routinely used for client submissions regardless of the PRRSV on
137 farm.

138

139 *2.7. RNA extraction and real-time PCR*

140 Total nucleic acids from serum and nasal swabs were extracted using the KingFisher Flex
141 96-tip comb (Thermo Scientific) and the MagMax-96 viral RNA isolation kit (Life
142 Technologies) [13]. Positive and negative controls were included on each plate. Extracted
143 samples were tested by quantitative reverse transcriptase (RT) PCR for the presence and amount
144 of PRRSV RNA [14]. The threshold was set at 0.05 with a cycle threshold (C_T) of less than 37
145 cycles considered positive.

146

147 *2.8. Characterization of the PRRSV strains detected by differential real-time RT-PCR*

148 To differentiate the vaccine candidate SAVE5 and the wild type PRRSV VR2385, a
149 duplex differential real-time RT-PCR was established based on the alignment of GP5 gene. The
150 VR2385 primers and probe were PRRS2385F: *5'*-GTGCCCTGGCTGCGTTGAT-3',
151 PRRS2385R: 5'-CAACGATAGAGTCTGCCCTTAGTGTC-3', PRRSprob2385: FAM-5'-
152 CTTCGTCATTAGGCTTGCGAAGAATTGC-3'-BHQ1. The SAVE5 primers and probe were
153 PRRSSAVEF: 5'-GCTGATTTACAACTTGACGCTATGTG-3', PRRSSAVER: 5'-
154 GACAGGAAAAATGACAAAGCACTCG-3', PRRSprobSAVE: CAL-Fluor®-Orange-560-5'-
155 TAACGGTACCGACTGGCTTGCGAATAAG-3'-BHQ1. The real-time RT-PCR was carried
156 out in 96-well plates, with each reaction consisting of a total volume of 25 µl, containing 12.5 µl
157 TaqMan One-Step RT-PCR master mix reagent (Applied Biosystems), 6 µl RNA, 0.625 µl 40×
158 MultiScribe and RNase Inhibitor, 1 µl each of the two primers (10 µM), 0.5 µl probe (10 µM)
159 and 3.375 µl RNase-free water. Amplification reactions were performed using an Applied
160 Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems) under universal conditions:

30 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. A sample was considered negative if no signal was detected during 40 amplification cycles.

PRRSV ORF5 was sequenced from two PRRSV RT-PCR positive pigs in each of the VAC-IM-PRRSV, VAC-IN-PRRSV and the POS-CONTROL groups on D54. In addition, the SAVE5 vaccine strain and the VR2385 challenge strain used for inoculation were also sequenced. PRRSV ORF5 amplification was performed using primers GP5F (5′-ATGTTGGGGAAATGCTTGACCG-3′) and GP5R (5′-CTAAGGACGACTCCATTGTTCCG-3′) [13]. The PCR products were sequenced using a 3730xl DNA Analyzer at the Iowa State University DNA Facility, Ames, Iowa, USA.

2.9. Necropsy

All pigs were humanely euthanized on D54 by intravenous injection of pentobarbital sodium overdose (Fatal Plus®, Vortech Pharmaceuticals, LTD, Dearborn, Michigan, USA). A veterinary pathologist blinded to the treatment groups of the pigs scored and recorded the severity of macroscopic lung lesions for each pig. Specifically, macroscopic lung lesions were assessed to determine the percentage of the lung affected by pneumonia [12]. Five sections of lungs, tonsil and tracheobronchial lymph nodes were fixed in 10% neutral buffered formalin and routinely processed for further histopathological analysis.

2.10. Histopathology and immunohistochemistry

Microscopic lesions were assessed by a pathologist blinded to the treatment groups. Lung lesions were scored for severity of PRRSV induced interstitial pneumonia lesions ranging from

0=normal to 6=severe diffuse [15]. Each pig was scored by two different pathologists and the average score was determined and used for further analysis.

To assess presence and amount of PRRSV antigen, immunohistochemistry was conducted on sections of lung tissues [16]. Scores ranged from 0=no antigen detectable to 3=abundant antigen diffusely distributed. All slides were independently evaluated by two pathologists and the mean score was utilized.

2.11. Statistical analysis

Summary statistics were calculated for groups to assess the distributional property. Quantitative RT-PCR data was log transformed prior to analysis. A generalized linear mixed model was used for all statistical comparisons with SAS version 9.3 (SAS Institute, Cary, NC) with group, time and their interaction were fixed effects and pig as random effect. A P-value of less than 0.05 was considered significant. Difference in mean response was assessed between groups. Protection was assessed by regrouping pigs in the VAC-IM-PRRSV and VAC-IN-PRRSV groups by seroconversion to PRRSV (S/P ratio greater than 0.4) at challenge (D42) into VAC-D42-POS (n=10) and VAC-D42-NEG (n=20) pigs (Table 2). These two groups were directly compared to the POS-CONTROLS. Area under the curve viremia (AUC) was calculated for each pig from D45 to D54. Correlation between AUC and antibody levels at challenge (D42) was assessed by Pearson's correlation test.

3. Results

3.1. Clinical signs and weight gain

205 The NEG-CONTROL, the VAC-IM-PRRSV and the VAC-IN-PRRSV groups had no
206 clinical signs of disease during the experiment. One POS-CONTROL pig had bilateral mucous
207 discharge starting at D44 with a respiratory score of 2 by D53. Two other POS-CONTROL pigs
208 had mucous discharge on D53. Statistical evaluation of the respiratory scores (data not shown) or
209 the average daily weight gain (ADG, Tables 1 and 2) indicated no significant differences among
210 the treatment groups. Differences in rectal temperatures were observed on D46 when the POS-
211 CONTROL group had statistically ($P < 0.05$) higher rectal temperatures compared to VAC-IN-
212 PRRSV and NEG-CONTROL groups.
213
214 *3.2. Seroconversion to PRRSV*
215 Mean group anti-PRRSV IgG antibody levels are summarized in Fig. 1. None of the pigs
216 had detectable PRRSV antibodies on arrival and the NEG-CONTROL group remained
217 seronegative until termination of the study. Vaccinated pigs developed anti-PRRSV IgG
218 antibodies starting at D14 and VAC-IN-PRRSV pigs had significantly higher S/P ratios
219 compared to pigs vaccinated intramuscularly at D28 (Table 3). Among vaccinated and
220 subsequently challenged pigs only 10/30 pigs (4/20 VAC-IM-PRRSV and 6/10 VAC-IN-
221 PRRSV) pigs had seroconverted by D42 (VAC-D42-POSITIVE). There was no evidence of
222 seroconversion in the remaining 20/30 pigs (VAC-D42-NEGATIVE; 16/20 VAC-IM-PRRSV
223 and 4/10 VAC-IN-PRRSV).
224 Neutralizing antibodies against PRRSV type 2 strains ISU-P or VR2332 were not
225 detected in any of the serum samples collected at D42.
226
227 *3.3. PRRSV viremia and shedding*

Efficacy of the SAVE approach in protecting against PRRSV

The NEG-CONTROL pigs remained PRRSV RNA negative throughout the study. PRRSV VR2385 was not detected prior to D42 in any vaccinated pig and PRRSV SAVE5 was never detected in POS-CONTROL pigs based on differential PCR and sequencing. In vaccinated pigs, SAVE5 RNA in serum samples was first detectable by D7 (Table 4). At the time of PRRSV challenge on D42, 22.5% (9/40) of the vaccinated pigs were SAVE5 viremic. After challenge, only VR2385 was detected in the VAC-IM-PRRSV group and in 3/5 viremic VAC-IN-PRRSV pigs; however, mixed SAVE5/VR2385 was detected in 2/5 viremic VAC-IN-PRRSV pigs. Prevalence rates and PRRSV loads were lower in VAC-IN-PRRSV pigs compared to POS-CONTROLS which was significant on D51 (Table 4).

When the D42 seropositive and seronegative subgroups were analyzed, VAC-D42-POS pigs had evidence of SAVE-replication prior to challenge whereas VAC-D42-NEG pigs did not (Fig. 2). Significant differences in amounts of VR2385 PRRSV viremia after challenge indicate that VAC-D42-POS pigs but not VAC-D42-NEG pigs were protected from VR2385 challenge (Fig. 2). There was a high correlation between AUC viremia and levels of antibody at challenge (r = -0.84 [CI -0.90, -0.73], p <0.0001) indicating that the presence of antibodies at challenge was correlated with lack of viremia after challenge.

Nasal SAVE5 shedding was not detected in any of the vaccinated pigs prior to challenge. After challenge, nasal VR2385 shedding was observed in 25% (D46), 35% (D48) and 15% (D50) of the VAC-IM-PRRSV pigs; in 40% (D48) of the VAC-IN-PRRSV pigs; and in 47.3% (D46 and D48), 10.5% (D50) and 5.3% (D53) of the POS-CONTROLS. The group mean $\log_{10}$ PRRSV genomic copy numbers in nasal swabs ranged from 3.3 to 6.8. Nasal VR2385 shedding was not detected in any of the VAC-D42-POS pigs whereas it was observed in 55% (11/20) of

the VAC-D42-NEG pigs for 1-4 consecutive days and in 47.3% (9/19) of the POS-CONTROL pigs for 1-7 consecutive days.

3.4. Macroscopic lesions

Gross lesion scores are summarized in Tables 1 and 2. Multifocal mottled tan areas of consolidation were found throughout the lungs of selected pigs in all PRRSV VR2385-infected groups. Challenged groups had significantly more severe lesions than the non-challenged groups ($P < 0.01$). The mean macroscopic lung lesions in VAC-D42-POS pigs were not significantly different from VAC-D42-NEG pigs but were significantly lower compared to the POS-CONTROL (Table 2).

3.5. Microscopic lesions

When observed, lung lesions were characterized by mild-to-moderate multifocal lymphohistiocytic interstitial pneumonia with type 2 pneumocyte hypertrophy and hyperplasia, and increased numbers of macrophages and necrotic cellular debris in the alveolar spaces. Group mean interstitial pneumonia scores are summarized in Table 1. Overall, pigs in the VAC-IN-PRRSV group had significantly less severe microscopic lesions compared to the POS-PRRSV group. When the D42 seropositive and seronegative subgroups were analyzed, VAC-D42-POS pigs had significantly lower scores compared to VAC-D42-NEG pigs and POS-CONTROL pigs (Table 2).

PRRSV antigen was observed in alveolar and septal macrophages in several pigs in the PRRSV-infected groups. The amounts of detectable PRRSV antigen in VAC-IN-PRRSV pigs were significantly lower compared to the POS-CONTROL pigs (Table 1). Among VAC-D42-

273 POS pigs, 3/10 were PRRSV IHC positive with significantly lower amounts of PRRSV antigen
274 in lung tissues compared to VAC-D42-NEG pigs and POS-CONTROL pigs (Table 2).
275
276 **4. Discussion**
277 The SAVE approach to attenuate viruses requires significantly less time compared to the
278 traditional cell culture attenuation, and most importantly the SAVE approach attenuates the virus
279 without altering the antigenicity of the virus protein on the virion, since the protein sequence of
280 the SAVE5 ORF5 is identical to the wild-type PRRSV ORF5. The attenuation is achieved by
281 modification of the naturally optimized pairs of codons in a viral gene sequence without altering
282 the codon bias or the amino acid sequence [17]. A potential drawback of the SAVE approach is
283 over-attenuation which may affect the ability of the virus to replicate in the host. In contrast, the
284 traditional cell culture back passage approach to attenuate viruses may introduce critical
285 mutations in the virus genome during serial passages in cell culture, and these mutations, often
286 only a few critical amino acid changes, contribute to virus attenuation but can also revert back to
287 pathogenic phenotype. Therefore, there are pros and cons for both approaches.
288 In this study, the vaccine efficacy of the PRRSV vaccine candidate SAVE5 was tested *in*
289 *vivo* using the homologous wild-type PRRSV strain VR2385. Similar to a previous pilot study,
290 SAVE5 virus proved to be attenuated. After vaccination, SAVE5 viremia was sporadic (< 50%
291 of the vaccinated pigs for 1-5 consecutive weeks) and of low magnitude, SAVE5 nasal shedding
292 was not detectable and clinical signs were absent. This further documents that codon-pair de-
293 optimization is an effective way to attenuate PRRSV. A farm-specific attenuated PRRSV
294 vaccine could be produced via the SAVE approach in less than 2 months from PRRSV positive
295 lung tissue or serum obtained from the farm.

Efficacy of the SAVE approach in protecting against PRRSV

In vaccinated pigs in this study, seroconversion was initially observed by D14 similar to that previously reported [11]. At challenge at D42, 30% (9/30; intramuscular route) to 60% (6/10; intranasal route) of the vaccinated pigs had detectable anti-PRRSV-IgG levels, and 20% (6/30; intramuscularly route) to 30% (3/10; intranasal route) were SAVE5 viremic. It is possible that the SAVE5 vaccine virus was too attenuated to replicate and elicit an antibody response in all of the pigs or that the vaccine virus dose used for vaccination was too low. To account for this, vaccinated pigs were further divided into VAC-D42-POS and VAC-D42-NEG groups.

It is well accepted that antibodies detected in the serum shortly after infection do not necessarily correlate with protection, and that rapid induction of neutralizing antibody provides vaccine-based protection against infection [8;18]. However, a neutralizing antibody response was not detected in the present study. This may be due to antigenic differences between the viruses used in the FFN assay and the actual SAVE5 strain used. The percentage of amino acid identity of VR2385 was 92.5% for ISU-VDL strain ISU-P and 91% for the VR2332. It may also provide further evidence that detectable neutralizing antibodies may not be an ideal correlate of protection against PRRSV. Cell-mediated immunity is considered more important than neutralizing antibodies in conferring protection against PRRSV, and PRRS-specific T-cells can be observed as early as 2 weeks after infection [19]. In this pilot study cellular immunity was not assessed due to limited access to necessary reagents; however, cellular immunity needs to be addressed in future studies. In general, the best measurement of protective immunity triggered by an effective vaccine is in a challenge infection model [18]. Interestingly, VR2385 viremia and nasal shedding after challenge was not detectable in VAC-D42-POS pigs which all had seroconverted by the time of challenge. This could indicate that vaccine-induced seroconversion is associated with protection.

Under the study conditions, the nasal vaccination route appeared to be more effective resulting in protective immunity in a larger number of pigs compared to the intramuscular route. The first pig with a SAVE5 viremia was detected by D7 in the VAC-IN-PRRSV group whereas the first SAVE5 viremic VAC-IM-PRRSV pig was detected by D14. At the day of challenge 60% (6/10) of the VAC-IN-PRRSV pigs had seroconverted to PRRSV in contrast to 20% (4/20) of the VAC-IM-PRRSV pigs indicating an earlier and more efficient induction of a low grade SAVE5 viremia with subsequent development of an adaptive immune response after administration of the SAVE5 vaccine strain by the intranasal route. Determination of possible differences between intramuscular and intranasal vaccination by assessing local mucosal immunity needs to be done in future.

The VAC-D42-POS pigs were protected from subsequent VR2385 challenge as evidenced by lack of VR2385 viremia, VR2385 nasal shedding, significantly reduced macroscopic and microscopic lung lesions and significantly reduced amounts of PRRSV antigen in lungs compared to the non-vaccinated POS-CONTROL pigs. This indicates that the SAVE vaccine strategy may be a feasible alternative in rapidly producing a farm-specific autogenous vaccine if the immunogenicity of the SAVE vaccine can be improved. Additional experimental studies using larger number of pigs, a higher vaccine virus dose, different challenge time point post-vaccination, and heterologous PRRSV strains including concurrent infections are needed to better evaluate the benefits of this novel vaccine approach.

5. Conclusions

The present study further confirmed that the SAVE approach can effectively attenuate a PRRSV strain. Additional work needs to be done to further improve SAVE5 vaccine efficacy.

342 The ability to utilize the SAVE technology to rapidly produce, safe and efficacious, farm-
343 specific PRRSV vaccines is practical and could have a major impact on reducing the major
344 economic losses associated with PRRSV.

345

346 **Funding**

347 Danielle Evenson was supported through the Iowa Livestock Health Advisory Council (ILHAC)
348 and the Iowa State University College of Veterinary Medicine Summer Scholars Research
349 Program. Funding for this study was provided by ILHAC and the Biotechnology and Biological
350 Sciences Research Council (BBSRC) Institute Strategic Programme Grant awarded to the Roslin
351 Institute (BB/J004324/1; BBS/E/D/20241864).

352

353 **Conflict of Interest**

354 The authors declare no financial and personal relationships with other people or organizations
355 that could inappropriately influence this work.

356

357 **Acknowledgments**

358 The authors thank Rachel Brooks, Brett Burke and Dr. Anil Thachil for assistance with the
359 animal work.

360

361 **References**

362

363 [1] Corzo CA, Mondaca E, Wayne S, et al. Control and elimination of porcine reproductive
364 and respiratory syndrome virus. Virus Res 2010 Dec;154(1-2):185-92.

[2] Holtkamp DJ, Kliebenstein JB, Neumann EJ, et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J Swine Health Prod 2013;21(2):72-84.

[3] Benfield DA, Nelson E, Collins JE, et al. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J Vet Diagn Invest 1992 Apr;4(2):127-33.

[4] Cavanagh D. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch Virol 1997;142(3):629-33.

[5] Snijder EJ, Kikkert M, Fang Y. Arterivirus molecular biology and pathogenesis. J Gen Virol 2013 Oct;94(Pt 10):2141-63.

[6] Nelsen CJ, Murtaugh MP, Faaberg KS. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J Virol 1999 Jan;73(1):270-80.

[7] Murtaugh MP, Stadejek T, Abrahante JE, Lam TT, Leung FC. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. Virus Res 2010 Dec;154(1-2):18-30.

[8] Hu J, Zhang C. Porcine reproductive and respiratory syndrome virus vaccines: current status and strategies to a universal vaccine. Transbound Emerg Dis 2014 Apr;61(2):109-20.

[9] Li X, Galliher-Beckley A, Pappan L, et al. Comparison of host immune responses to homologous and heterologous type II porcine reproductive and respiratory syndrome virus (PRRSV) challenge in vaccinated and unvaccinated pigs. Biomed Res Int 2014;2014:416727.

[10] Opriessnig T, Baker RB, Halbur PG. Use of an Experimental Model To Test the Efficacy of Planned Exposure to Live Porcine Reproductive and Respiratory Syndrome Virus. Clin Vaccine Immunol 2007 Dec 1;14(12):1572-7.

[11] Ni YY, Zhao Z, Opriessnig T, et al. Computer-aided codon-pairs deoptimization of the major envelope GP5 gene attenuates porcine reproductive and respiratory syndrome virus. Virology 2014 Feb;450-451:132-9.

[12] Halbur PG, Paul PS, Frey ML, et al. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet Pathol 1995 Nov;32(6):648-60.

[13] Gerber PF, O'Neill K, Owolodun O, et al. Comparison of commercial real-time reverse transcription-PCR assays for reliable, early, and rapid detection of heterologous strains of porcine reproductive and respiratory syndrome virus in experimentally infected or noninfected boars by use of different sample types. J Clin Microbiol 2013 Feb;51(2):547-56.

401 [14] Sinha A, Lin K, Hemann M, et al. ORF1 but not ORF2 dependent differences are
402 important for in vitro replication of PCV2 in porcine alveolar macrophages singularly or
403 coinfected with PRRSV. Vet Microbiol 2012 Jul 6;158(1-2):95-103.

404 [15] Thacker EL, Halbur PG, Ross RF, Thanawongnuwech R, Thacker BJ. Mycoplasma
405 hyopneumoniae potentiation of porcine reproductive and respiratory syndrome virus-
406 induced pneumonia. J Clin Microbiol 1999 Mar;37(3):620-7.

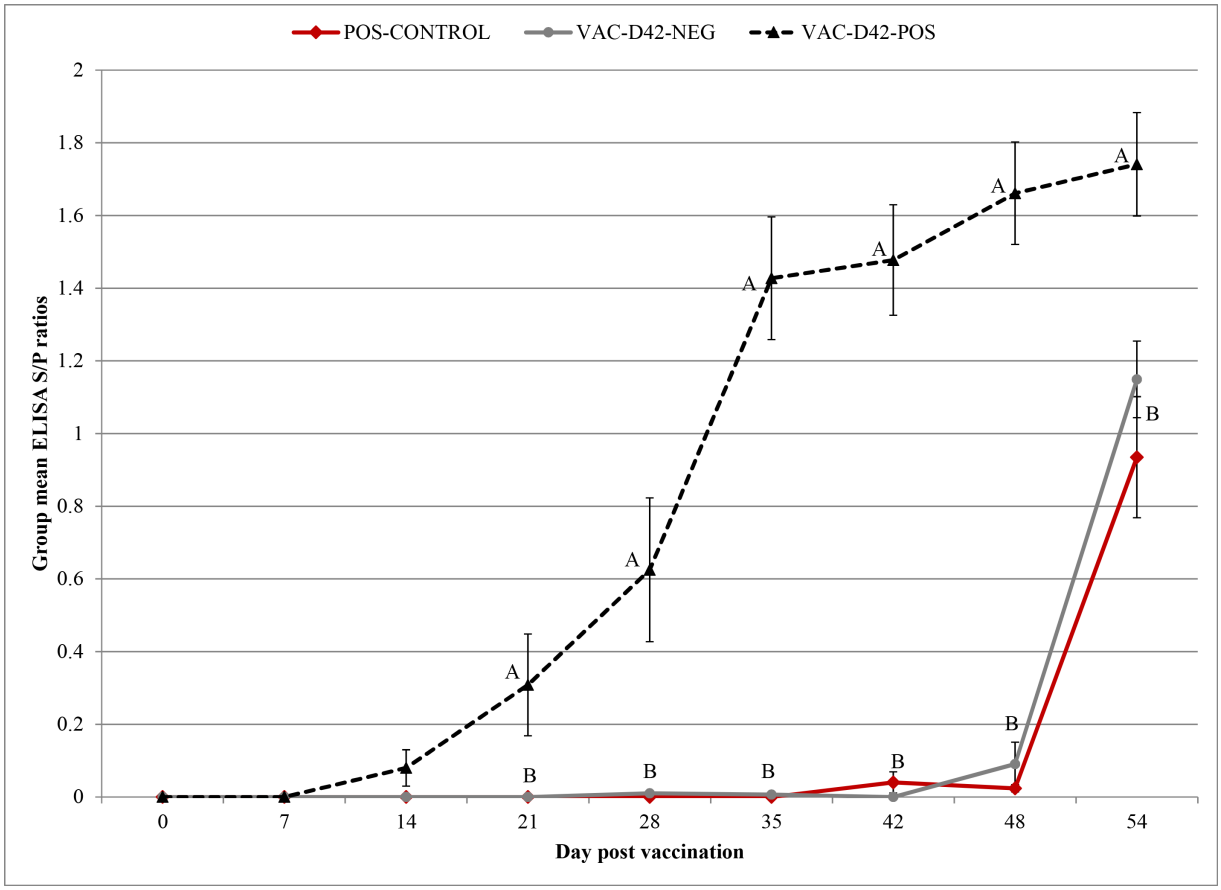
407 [16] Halbur PG, Andrews JJ, Huffman EL, Paul PS, Meng XJ, Niyo Y. Development of a
408 streptavidin-biotin immunoperoxidase procedure for the detection of porcine reproductive
409 and respiratory syndrome virus antigen in porcine lung. J Vet Diagn Invest 1994
410 Apr;6(2):254-7.

411 [17] Mueller S, Coleman JR, Papamichail D, et al. Live attenuated influenza virus vaccines by
412 computer-aided rational design. Nat Biotechnol 2010 Jul;28(7):723-6.

413 [18] Loving CL, Osorio FA, Murtaugh MP, Zuckermann FA. Innate and adaptive immunity
414 against Porcine Reproductive and Respiratory Syndrome Virus. Vet Immunol
415 Immunopathol 2015 Sep 15;167(1-2):1-14.

416 [19] Xiao Z, Batista L, Dee S, Halbur P, Murtaugh MP. The level of virus-specific T-cell and
417 macrophage recruitment in porcine reproductive and respiratory syndrome virus infection
418 in pigs is independent of virus load. J Virol 2004 Jun;78(11):5923-33.
419
420

421 **Figure Legends**



422

423 **Fig. 1.** Group mean ELISA sample-to-positive (S/P) ratios over time in POS-CONTROL pigs
424 (n=19), VAC-D42-NEG pigs (n=20) and in VAC-D42-POS pigs (n=10). Pigs were vaccinated at
425 D0 and inoculated with PRRSV on D42 (arrow). An S/P ratio greater than 0.4 was considered
426 positive. Different superscripts (^{A,B}) indicate significantly ($P < 0.05$) different group means for a
427 certain day.

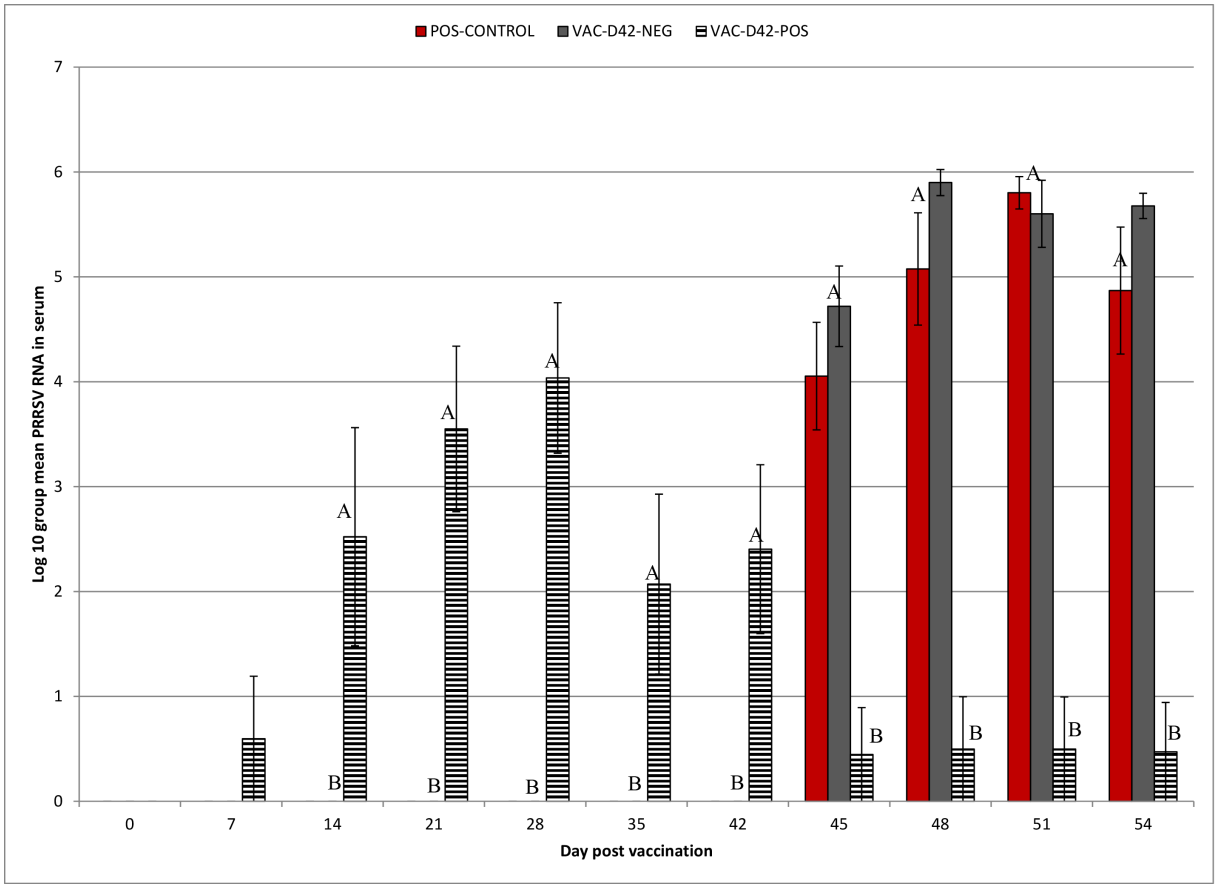


Fig. 2. Group mean $\log_{10}$ PRRSV genomic copies per ml serum over time in POS-CONTROL pigs (n=19), VAC-D42-NEG pigs (n=20) and in VAC-D42-POS pigs (n=10). Pigs were vaccinated at D0 and SAVE5-PRRSV and inoculated with PRRSV on D42. Different superscripts (^{A,B}) indicate significantly ($P < 0.05$) different group means for a certain day.